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Journal of Crop Improvement

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title-content=t92303981>

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Online Publication Date: 01 July 2009

To cite this Article Glynn, Neil C., Gilbert, Robert A., Glaz, Barry, Comstock, Jack C., Kang, Manjit S., Deren, Christopher W., Tai, Peter Y. P. and Miller, Jimmy D. (2009) 'Repeatability Between Two Intermediate Sugarcane Genotype Selection Stages in Florida', *Journal of Crop Improvement*, 23:3, 252 — 265

To link to this Article: DOI: 10.1080/15427520902805290

URL: <http://dx.doi.org/10.1080/15427520902805290>

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Repeatability Between Two Intermediate Sugarcane Genotype Selection Stages in Florida

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Improved yield and disease resistance on sand soils are priorities of the Canal Point (CP) sugarcane (Saccharum spp.) breeding and selection program. Analyses of historical phenotypic data can provide helpful information in guiding selection strategies to meet these priorities. Correlation analysis was used to examine repeatability of phenotypic data used to advance genotypes from an unreplicated single location clonal crop test (stage II) to the subsequent stage (stage III; two replicate, four location clonal crop experiment). Correlations between data for four traits measured in stage II and the corresponding data pooled across soil types for the same genotypes in stage III varied across 23 series of the CP program. Generally, when correlations were statistically significant ($P < 0.05$), correlation values were low (means; theoretical recoverable sucrose (TRS) $r = 0.40$, cane yield $r = 0.27$, and economic index $r = 0.23$). Similar trends were evident for correlations between data from stage II and stage III on muck soil and stage II and stage III on sand soil across 10 series of the CP program. A 10%

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We are indebted to Sarah Lingle, Ron Rice, and James Shine, Jr. for helpful comments and suggestions on an early draft of this manuscript. Product names and trademarks are mentioned to report factually on available data; however, the USDA-ARS neither guarantees nor warrants the standard of the product, and the use of the name by USDA-ARS does not imply approval of the product to the exclusion of others that may also be suitable. The experiments reported comply with current US laws.

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reduction in the number of genotypes advanced to stage III over that period would have meant losing only 1 and 13 genotypes that had commercial potential on muck and sand soils, respectively ($n = 1278$). Correlations between the phenotypic data were significant only for stage III comparisons between TRS and cane yield, which were negatively associated on either soil type. These results indicate that changes in the advancement strategy from stage II are not required as advancing approximately 135 genotypes identifies almost all genotypes with the genetic potential to yield well on muck or sand soils in stage III. Increasing genotypes in stages prior to stage III and changing crossing strategies to improve identification of disease-resistant, high-yielding genotypes for sand soils is recommended.

KEYWORDS sugarcane breeding, cane yield, sucrose yield, Histosol, muck soil, sand soil

INTRODUCTION

The Canal Point sugarcane cultivar development program (CP program) follows a protocol of genotype advancement involving single replicate, single site experiments in the early stages: seedlings (~100,000 genotypes) and stage I (~15,000 genotypes). The final stage, stage IV, has six replicates, 10 locations, 13 new genotypes per location, and three crop cycles (plant cane, first ratoon, and second ratoon). For review of the CP program, see Miller (1994). Stage II (unreplicated, single location ~1500 genotypes) and stage III (two replicates, four locations, ~135 genotypes) are the first in which quantitative yield data are used for genotype advancement. Stages II and III are the intermediate stages between early, mass selection in seedlings and stage I, where advancement is based primarily on visual selection and the more rigorous evaluations of genotypes with commercial potential in stage IV of the CP program. The genotypes advanced to stage III are generally those with adequate disease resistance and the highest values for cane yield, sucrose yield, theoretical recoverable sucrose (TRS) or sucrose content, and economic index (EI). The economic index includes theoretical costs and profits related to hauling and processing stalks with differing values of cane yield and TRS (Deren, Alvarez, & Glaz, 1995). Although this general procedure (except for the use of EI) has been in place since the mid-1960s, little is known about repeatability between stages II and III.

The CP program produces improved cultivars primarily for the growing conditions of South Florida. The two major soil types on which sugarcane is produced in South Florida are Histosols (muck soils) and Entisols or Spodosols (sand soils). Histosols have organic matter greater than 20%–30%, but most of the muck soils in south Florida are much higher, sometimes reaching

85% organic matter (Snyder, 1994). The sand soils have <3% organic matter. Edmé et al. (2005) reported that from 1968 to 2000 mean yearly increases on muck soils in sucrose content (0.80 kg Mg^{-1}), cane yield (0.31 Mg ha^{-1}), and sugar yield (0.10 Mg ha^{-1}) were attributable to genetic improvements resulting from the CP program. However, there was no genetic improvement for sugarcane grown on sand soils. This finding was suggested as being due to the early stages of the CP program being performed exclusively on muck soils and therefore ineffective at identifying genotypes adapted to sand soils. The authors suggested that testing genotypes on sand soils in the seedling, stage I, and stage II of the CP program would improve the identification of high-yielding genotypes for sand soils. In the current CP program, genotypes are evaluated on a sand soil for the first time at one of the four stage III locations, the other three being muck soils. A key hypothesis to be tested to determine the need for a stage II trial on sand soils (as suggested by Edmé et al., 2005) is how repeatable is the muck stage II to the muck stage III tests and to the sand stage III test.

Genotypes are not usually advanced from stage II if one or more of several diseases are observed. However, in the case of sugarcane brown rust, caused by *Puccinia melanocephala*, high-yielding genotypes that show low susceptibilities are often advanced. A recent race shift in the brown rust pathogen impacted cultivar LCP 85-384 (Hoy, 2005), which accounted for more than 90% of the sugarcane acreage in Louisiana. This along with the recent first report in Florida and the western hemisphere of sugarcane orange rust caused by *P. kuehnii* (Comstock et al., 2008) have highlighted the importance of rusts to the U.S. sugarcane industry. Sugarcane orange rust is now also present in Guatemala (Ovalle et al., 2008), Nicaragua, and Costa Rica (Chavarría et al., 2008). These industries grow cultivars from the CP program and use CP material as parents in their crossing programs. It would be desirable to advance to stage III only genotypes that are completely free from rust. However, such a strategy may result in advancing too many genotypes with unacceptably low yields. Assessing genotype repeatability between stage II and stage III may help resolve this issue. If genotype yields between the two stages are not highly repeatable, then placing more emphasis on rust resistance and less emphasis on yield in stage II may more effectively identify high-yielding genotypes with commercial potential.

Several previous studies have examined repeatability within sugarcane breeding programs. Sucrose yield was shown to be highly repeatable between crop years (plant cane and first ratoon) by Jackson (1992), and additionally cane yield and sucrose content were shown to be repeatable by Tai and colleagues (1980) and by Mamet and Domaingue (2001). Repeatability estimates have also been made between sugarcane selection stages; genotype correlations between stages III and IV for sucrose yield and EI were significant but low in 24 cycles of the CP program (Glaz et al., 2002). However, Glaz et al. (2002) did not separate genotype repeatability by

environment. Repeatability of data generated between stage II and stage III was examined by Tai et al. (1980). Although they reported stalk number, stalk weight, Brix, sucrose percentage, and sugar per ton of cane as highly repeatable, and cane and sucrose yields as not repeatable, their findings were based on data from only 93 genotypes within one series (year) of the CP program. Falconer (1966) defined estimates of repeatability made on the same genotypes in different locations as clonal repeatability (r_c). Kang, Miller, and Tai (1984) examined (r_c) for several yield related traits between the four stage III locations of the CP program. They showed that (r_c) was generally greater for sucrose content than cane yield and sucrose yield, respectively, between data from 105 genotypes tested at four stage III locations in the CP program. Understanding associations between selection data can provide useful guides to selection strategies and the allocation of resources. The literature is conflicting on the existence of correlations between the two main criteria used for sugarcane selection—sucrose content and cane yield. A negative correlation between cane yield and sucrose content was reported by Kang, Miller, and Tai (1983), and by Milligan et al. (1990), and between sucrose content and stalk weight, stalk diameter, and stalk length by Gravois and Milligan (1992). In contrast, Alvarez, Deren, and Glaz (2003) did not find a consistent negative relationship between sucrose content and cane yield among 164 genotypes from the final stages of the CP program across 20 years. Jackson (1994) reported a positive correlation between sucrose content and cane yield in plant cane of sugarcane genotypes with a large component of *Saccharum spontaneum*, but a small negative correlation in the first and second ratoon crops. In this investigation, we expand the definition of clonal repeatability defined by Kang, Miller, and Tai (1984) to also include comparisons made on the same genotypes between selection stages, in this case stages II and III of the CP program.

The objectives of this study were to: 1) determine genotype repeatability of cane yield, TRS, sucrose yield, and EI between stages II and III of the CP sugarcane cultivar-development program; 2) determine correlations among those data; and 3) examine the effect of soil type on repeatability between the two selection stages. It was hoped that pursuing these objectives would help determine whether the CP program should add a stage II trial on a sand soil and whether selection in stage II should place more emphasis on rust resistance while proportionately reducing emphasis on yield to improve the probabilities of identifying rust-resistant genotypes with high yields for sand soils.

MATERIALS AND METHODS

Source of Data

The data set in this study consisted of stages II and III for 23 consecutive series (annual selection cycles) of the CP program from 1984 (CP 82 series)

through 2007 (CP 04 series). The number of genotypes in each series was generally near 135; although for the CP 83 series, data were available for only 27 genotypes (Table 1). The traits evaluated were cane yield (Mg ha^{-1}), TRS (g kg^{-1}), sucrose yield (Mg ha^{-1}), and EI from a total of 2788 genotypes evaluated in plant cane stage II. The same traits were also evaluated in 23 stage III plant-cane crops, and 834 of the 2788 genotypes were evaluated in 23 stage III first-ratoon crops. A detailed description of the test procedures employed in the CP program is given in Glaz et al. (2008). Briefly, stage II trials were planted at Canal Point, Florida, and consisted of one plot for each genotype. At each of the four stage-III locations, advanced genotypes were replicated twice in randomized complete block designs. Plots were the same size in stage II and stage III: two rows were 1.5 m apart and 4.6 m long. Stage III experiments were performed at the same four south Florida locations each year: A. Duda and Sons Inc. (DU), Hilliard Brothers (HI), Okeelanta Corporation (OK), and South Florida Industries (SF). Planting dates varied among years from September to December, but the majority

TABLE 1 Number of Genotypes Used as a Source of Stage II (Plant Cane), Stage III Plant Cane, and Stage III First Ratoon Yield Data in Canal Point Sugarcane Genotype Trials from 1982–2004

CP series	Number of genotypes	
	Stage II and stage III plant cane	Stage III first ratoon
82	102	27
83	27*	27
84	102	33
85	103	40
86	132	40
87	131	41
88	131	40
89	130	37
90	130	39
91	132	39
92	135	40
93	124	40
94	131	40
95	122	38
96	135	40
97	132	40
98	134	40
99	129	40
00	135	38
01	128	38
02	135	21
03	103	19
04	125	37

*CP 83 series plant cane data were only available for those genotypes advanced to the first ratoon trials.

were in November and December. In October of each year, a 10 stalk sample from each plot was weighed and milled to extract the juice. The sample weight, together with the total number of stalks counted in each plot (usually in August), was used to calculate cane yield (Mg ha^{-1}). In each year, Brix and pol were measured in the extracted juice. These data were used to calculate TRS as grams of sucrose per kilogram of cane according to methods described by Legendre (1992). Values for cane yield and TRS were used to calculate sucrose yield (Mg ha^{-1}), and EI was subsequently calculated according to Deren, Alvarez, and Glaz (1995). For stage III genotypes, a mean value across replicates and locations was generated for each trait of each genotype (Table 1). Due to flooding, there were no data for plant cane of the CP 99 series at SF.

Repeatability Between Stage II and Stage III and Correlations Between Characters

Data repeatability was examined by correlation analysis. The correlations of cane yield, TRS, sucrose yield, and EI from the unreplicated stage II experiment with the same data from the same genotypes in stage III plant cane and stage III first ratoon, and between the plant cane and first ratoon crop cycles in stage III, were determined separately for each of the 23 series using PROC CORR (SAS Institute, 2003). Data from stage III was the mean across replicates and locations for each of the four traits.

Data collected from stages II and III for 10 consecutive annual series of the CP breeding program between 1997 (CP 95 series) and 2007 (CP 04 series) were analyzed to estimate repeatability between stage II trials on muck and stage III trials on muck and stage II trials on muck and stage III trials on sand soils. Mean values for cane yield, TRS, sucrose yield, and EI were generated separately for stage III trials planted on muck soil locations (DU, OK, and SF) and the sand soil location (HI). For each series, the correlation between stage II and stage III (plant cane) was performed separately using stage III data from sand soils and muck soil locations using PROC CORR (SAS Institute, 2003).

Relative changes in individual genotype performance between stage II and stage III trials on muck and sand soils were determined for the four traits of interest. Genotypes were ranked according to cane yield, TRS, sucrose yield, and EI (in descending order) in stage II. Genotypes were identified that ranked within the top 5%, 10%, and 20% of genotypes (three top tiers) in stage II for each of the four characters. Similarly, genotypes were identified that ranked within the bottom 5%, 10%, and 20% (three bottom tiers) for these characters in stage II. Subsequently, the number of genotypes was identified for each character that switched from any of the top tiers in stage II to any of the bottom tiers in stage III, or from any of the bottom tiers in stage II to any of the top tiers in stage III.

The six possible correlation combinations between the four characters within each wtage for each of the 23 years were also determined using PROC CORR (SAS Institute, 2003). Correlations between cane yield and TRS were determined for the stage II data set for the CP 95 through CP 04 series, and separately for the same data derived from stage III across the muck-soil locations and the sand-soil location using PROC CORR (SAS Institute, 2003). For all analyses, significant differences were determined at $P < 0.05$.

RESULTS AND DISCUSSION

Overall Repeatability Between Stages II and III for 23 CP Series

Frequency distributions of the significant r -values for comparisons between stage II and stage III plant cane, stage II and stage III first ratoon, and stage III plant cane and stage III first ratoon for cane yield, TRS, sucrose yield, and EI are given in Figure 1. Overall, the significant r -values were highest for TRS (mean r value = 0.40), followed by cane yield (mean r value = 0.38), sucrose yield (mean r value = 0.27), and EI (mean r value = 0.23). The comparison between the two stage III crop cycles had the highest correlation (mean r value = 0.48), followed by stage II and stage III plant cane (mean r value = 0.29), and stage II and stage III first ratoon (mean r value = 0.19).

Correlations Between Characters

The correlation between cane yield and TRS was the only significant correlation among the six possible two-way comparisons between the four characters examined. A frequency distribution of the r -values for the correlation between cane yield and TRS from the stage II, stage III plant cane, and stage III first ratoon data sets for the 23 CP series shows that significant correlations were all negative (Figure 2). Using data from all 23 years, the negative correlations between cane yield and TRS were significant for stage III data from muck soils, stage III sand soils, and non-significant for stage II (Table 2). The implications for this finding to the CP program are that the selection of high yielding genotypes in stage II with high sucrose content is possible, and emphasis on either TRS or cane yield should not adversely affect the other. However, in stage III it will be more challenging to identify genotypes with high TRS and high cane yield. Miller and James (1975) reported a non-significant relationship between Brix and cane yield in stage II of the CP program among 376 clones from six families. Jackson (2005) suggested that the relationship between cane yield and sucrose is a reflection of a genotype's assimilation of carbon and the partitioning of photosynthate between growth and storage, and that this partitioning would act in opposite directions in most environments, resulting in genetic correlations between these measurements close to zero. The relationship was, however,

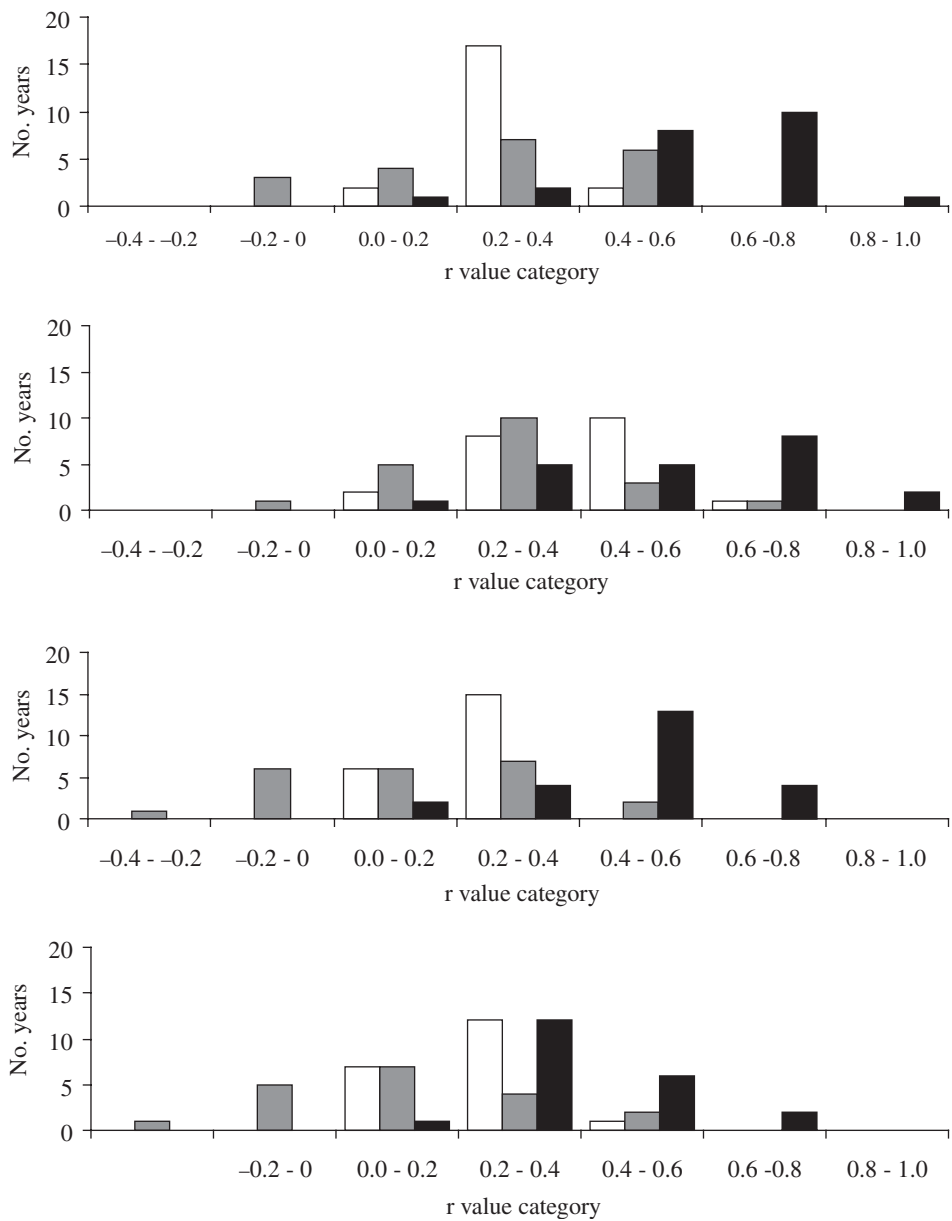


FIGURE 1 Frequency distributions of significant ($P < 0.05$) r -values for correlations in cane yield, theoretical recoverable sucrose (TRS), sucrose yield, and economic index (EI) between stage II and stage III plant cane, stage II and stage III first ratoon, and stage III plant cane and stage III first ratoon for 23 series of the Canal Point sugarcane cultivar development program.

specific to each population and sensitive to the environment and field-level competition effects. The difference in correlations between cane yield and TRS between stage II and the two stage III soil types supports the hypothesis of Jackson (2005) that the relationship is environment specific.

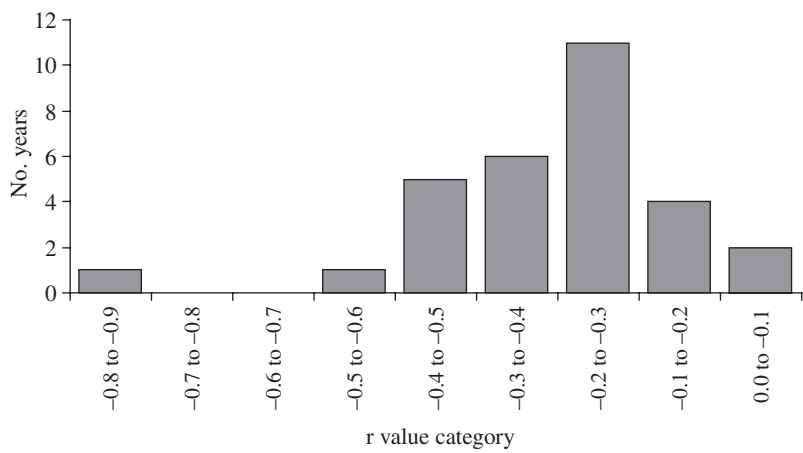


FIGURE 2 Frequency distribution of significant ($P < 0.05$) r -values for the correlations between cane yield and theoretical recoverable sucrose (TRS) for sugarcane genotypes in stage II, stage III (plant cane), and stage III (first ratoon) in 23 CP series (CP 82 to CP 04).

TABLE 2 Correlations Between Cane Yield and Theoretical Recoverable Sucrose for Stage II and the Same Genotypes in Stage III Trials on Muck Soils and a Sand Soil ($n = 1278$)

Parameter	Stage II ($n = 1278$)	Stage III muck soils ($n = 1278$)	Stage III sand soil ($n = 1278$)	Pooled ($n = 3834$)
Correlation	0.001	-0.267	-0.324	-0.284
P	0.98	<0.01	<0.01	<0.01

Repeatability of Stage II with Stage III on Muck and Sand Soil Types

Stage II was generally better correlated to stage III on the muck soils than on the sand soil from the 10 CP series examined, although all correlations were low (Table 3). The mean r -values were highest for the correlations between the two soil types in stage III for all four characters and generally lowest for the comparisons between stage II and stage III sand soils. Of the four characters examined, the r -values were highest for TRS in all three comparisons and generally lowest for EI (Table 3). These results indicate similar genotype adaptabilities to muck soils and sand soils. Similarly Kang, Miller, and Tai (1984) reported significant correlations (r_c) that were greatest for TRS and similar for cane yield and sucrose yield between data from one stage III sand location and that from three muck locations.

The adaptability of CP germplasm compared with genotypes from other sugarcane breeding programs was examined by Gilbert et al. (2007) in a comparison of yield and economic performance of 50 sugarcane genotypes from 11 different countries on the sand soils of Florida. Eleven of 13 genotypes with CP parentage were in the upper half of genotypes ranked

TABLE 3 Summary of Significant ($P < 0.05$) Correlations of Stage II with Stage III on Sand Soil, Stage II with Stage III on Muck Soils, and Between Stage III on Sand Soil and Stage III on Muck Soils for Cane Yield, Theoretical Recoverable Sucrose (TRS), Sucrose Yield, and Economic Index (EI) for Sugarcane Genotypes from 10 CP Series (CP 95 to CP 04)

Data	Stage II and stage III sand soil		Stage II and stage III muck soils		Stage III sand and stage III muck soils	
	Number of significant series	Mean significant r values	Number of significant series	Mean significant r values	Number of significant series	Mean significant r values
Cane yield	5	0.29	9	0.29	10	0.45
TRS	7	0.30	9	0.42	10	0.60
Sucrose yield	4	0.28	7	0.26	10	0.42
EI	4	0.27	7	0.29	10	0.45

by EI and the six most profitable genotypes were all from the CP program. The authors concluded that the unusually low organic matter in the Florida sand soils used to evaluate genotypes in the CP program may have accounted for their higher yields compared with exogenous genotypes, which were selected primarily on sand soils with higher clay contents. This broad adaptability may be the result of the exploitation of a diverse gene pool within the CP breeding program, a factor suggested by Edmé et al. (2005) as accounting for the lack of a yield plateau among CP cultivars.

Changes in Rankings Between Stage II and Stage III on Muck and Sand Soil Types

From the 1,278 genotypes examined, the number of genotypes that had substantial changes in rank position (low to high and high to low) at the 5%, 10%, and 20% levels between stages II and III was greater for stage III sand soil than stage III muck soils, except that there was one more genotype that moved from the highest to the lowest 5% for TRS from stage II to stage III muck than for stage II to stage III sand (Table 4). No genotypes moved from the lowest ranked 5% of genotypes in stage II to the highest 5% of genotypes on stage III muck soils for any of the three characters, and only one genotype made that transition at the 10% level for each character. During this 10 year period, only two genotypes for cane yield, two for TRS, and three genotypes for sucrose yield moved from the lowest ranked 5% in stage II to the highest ranked 5% in stage III on the sand soil (Table 4). For 24 CP series (CP 69 to CP 92) in stage III, a previous study examined the performance of sugarcane genotypes that ultimately became commercial cultivars (Glaz et al., 2002). Only one genotype that ranked below 15th in stage III for sucrose yield and EI became a commercial cultivar planted on more than 1% of Florida's sugarcane acreage. Combining our results with those of Glaz and colleagues (2002) revealed that a 10% reduction in the

TABLE 4 Number of Sugarcane Genotypes that Changed from Highest to Lowest or Lowest to Highest 5%, 10%, and 20% Rankings for Cane Yield, Theoretical Recoverable Sucrose (TRS), and Sucrose Yield Between Stage II and Stage III Trials on Muck and Sand Soils for 10 CP Sugarcane Series (CP 95 Through CP 04; n = 1278)

		Number of genotypes that changed ranking between stage II and stage III (n = 1278)					
Data	Group	From highest to lowest rank			From lowest to highest rank		
		Muck	Sand	Pooled	Muck	Sand	Pooled
Cane yield	5%	1	3	1	0	2	0
	10%	2	10	1	1	13	2
	20%	16	25	9	19	35	16
TRS	5%	2	1	2	0	2	0
	10%	3	5	4	1	6	3
	20%	13	38	16	9	34	20
Sucrose yield	5%	2	3	2	0	3	0
	10%	3	10	2	1	10	2
	20%	13	37	7	14	43	16

number of genotypes (approximately 14) that were advanced from stage II to stage III would not affect the ability of the CP program to identify commercial cultivars for muck or sand soils.

CONCLUSIONS

The first objective of this study was to examine the success of stage II in the CP program at identifying high-yielding genotypes for sand soils compared with muck soils. The motivation for this analysis was to assess the need for an additional stage II trial on a sand soil as a means of improving the ability of the CP program to identify high-yielding genotypes for sand soils. Despite poor genotype repeatability between Stages II and III, advancing approximately 135 genotypes from stage II to stage III has been an effective strategy at ensuring that any genotype with the genetic potential to yield well on muck or sand soils is advanced to stage III. A 10% reduction in the number of genotypes advanced to stage III would not substantially impact the ability of the CP program to identify commercial cultivars for both major soil types on which sugarcane is grown in Florida. Although a 10% reduction in the number of genotypes advanced to stage III would not represent a significant savings in resources for planting and sampling, the additional plots available resulting from such a reduction could be used to evaluate novel germplasm or germplasm with novel uses such as biofuels.

Although the results from this study suggest that a stage II experiment on sand is not required, a stage II trial on sand soil using the same genotypes as the stage II at Canal Point would provide a useful additional

method for determining differences in genotype selection for each environment. However, it has been shown that sugarcane experiments on sand soils are known to suffer from increased variability compared with experiments on muck soils (Glaz & Kang, 2008), a factor that is a major concern when selecting large numbers of genotypes from unreplicated genotypes planted in small plots as is done in stage II in the CP program.

A second motivation for this study was to determine strategies for reversing a multi-year trend of increased susceptibility to brown rust in the CP germplasm. Less than 20% of the genotypes in stage II were completely free of brown rust in 2007, and of those, only 10% had satisfactory yields for advancement to stage III (data not shown). Our results indicate that reducing emphasis on yield and increasing emphasis on resistance to brown rust in stage II would not improve the ability of the CP program to identify disease-free genotypes with commercial potential. While this strategy may improve resistance to brown rust, it would result in the advancement of more low-yielding genotypes from stage II. The lowest yielding of the 135 genotypes advanced from stage II rarely were high-yielding genotypes in stage III. Thus, reducing emphasis on yields in stage II and advancing lower-yielding stage II genotypes is not a promising strategy for identifying rust-resistant, high-yielding genotypes. It was hoped that low repeatability between stages II and III would encourage less emphasis on yield in stage II, but again, this low repeatability is effectively resolved by selecting 135 genotypes in stage II.

The results of this study suggest that the CP program focus on increasing the frequency of advancement into stage II of rust-resistant genotypes and genotypes with high yield potential on muck and sand soils. Doing so may require increasing the number of families and genotypes evaluated in early selection stages in the CP program, heritability studies to improve parental combinations that favor rust-resistant progeny—a strategy proposed by Tai, Miller, and Dean (1981)—or modifications to early-stage selection strategies that allow efficient screening of rust susceptibility.

Generally, breeding and selection strategies for improving productivity in a specific environment should involve selection in that environment. The development of cultivars for sand soils prior to stage III in the CP program does not strictly follow this strategy because selection in these early stages is conducted on muck rather than sand soils. However, climatic factors are similar among all locations (muck and sand soils) where commercial sugarcane is grown in Florida. Despite not conducting selections on sand soils, the data presented indicate that the selection of productive genotypes adapted for sand soils is similar to those for muck soils in stage II. This finding taken with the results of Edmé and colleagues (2005), who showed genetic gains for cultivars grown on muck and not sand soils, indicate that efforts to improve genetic gains on sand soils in the CP program should focus on those stages prior to stage II. These could include the identification

of characters that influence high productivity on sand soils followed by crossing strategies to favor these characters or increasing the number of genotypes examined in seedlings and stage I.

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